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Clinical Presentation and Diagnosis of Gastrointestinal Infections by *Yersinia enterocolitica* in 261 Dutch Patients

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A surveillance of the clinical manifestations, course and outcome of 261 patients with gastrointestinal infection by *Yersinia enterocolitica* between 1982 and 1991 was carried out. Acute uncomplicated enteritis was diagnosed in 169 patients, complicated enteritis in 37, appendicular syndrome in 33, ileitis in 8 and colitis in 14. Children (age <16 years, $n=105$) presented most often with mild enteritis, young adults (age 16–25 years, $n=47$) with enteritis or appendicular syndrome, adults (age >25 years, $n=109$) had significant risk for developing serious enteritis, ileitis and colitis. Complications included reactive arthritis, septicaemia, lymphadenitis, disturbed liver functions and erythema nodosum. Four patients died of generalized peritonitis. Diagnosis was established by positive culture in 207 patients. Another 54 patients were diagnosed by having at least two other positive tests: serum agglutinins, specific IgA and IgG antibodies to *Yersinia* outer membrane proteins (Yops) or antigen detection in biopsies. Culture alone was sufficient to diagnose uncomplicated enteritis, antiYops serology appeared to be very useful in diagnosing patients with other manifestations of yersiniosis. The majority of the infections were caused by serotypes O3 and O9 while unusual serotypes were associated with advancing age and colitis.

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INTRODUCTION

Yersinia enterocolitica is a Gram-negative pathogen, associated with a wide spectrum of enteric and systemic manifestations (1). Diagnosis is not easy because the disease has diverse clinical presentations and stool cultures require specific laboratory methods, most of which are not performed routinely. Furthermore, stool cultures and serology by demonstration of circulating agglutinins are often negative in the more complicated forms (2, 3), and both may contribute to underestimation of the disease. In 1982 we began a study on *Yersinia* infections. At first all patients with enteritis were cultured for *Y. enterocolitica* and their physicians were asked to record all symptoms in cases with positive culture. They were also instructed to be alert for conditions commonly associated with *Y. enterocolitica* infections. When no material was available for culture the agglutination test was performed. In 1986 we introduced determination of serum IgA and IgG antibodies against *Yersinia* outer membrane proteins (Yops) (4) and antigen detection in biopsies (5). The *Yersinia* agglutination test is based on whole bacterial cells as antigens leading to cross-reactivity with antibodies directed against some *Brucella* spp. and *Enterobacteriaceae*. *Yersinia* virulence plasmids encoding for Yops have not yet been found in other *Enterobacteriaceae*, which makes this a more specific test than the agglutination test (6). IgA antibodies appear after about 10 days postinfection, followed by specific IgG antibodies. In an acute infection IgA reactivity decreases rapidly after 3–6 months, whereas a decrease of IgG reactivity is markedly retarded. In chronic infection persistent IgA and IgG reactivity is seen (7, 8). Antigen detection was

performed by the pathologist unaware of the results of culture or serology when biopsy specimens were available.

This study reports incidence and clinical manifestations of gastrointestinal infections by *Y. enterocolitica* during a 9-year study period.

MATERIALS AND METHODS

Culture

Fecal samples were inoculated onto *Yersinia* selective agar (Oxoid CM653, PCH-Diagnostica, Haarlem, The Netherlands), deoxycholate citrate agar (Oxoid CM35, PCH-Diagnostica) and into Rappaport broth. Plates were incubated at 22°C for 48 h; broth was incubated at 22°C for 72 h and then subcultured on the same plates. Colonies suspected to be *Yersinia* were isolated and identified (9). Other materials (pus, blood) were routinely cultured for aerobic and anaerobic bacteria and in particular for *Yersinia* on selective agar and in Rappaport broth, incubated at 22°C for 72 h. Pus was also stored for cold enrichment in phosphate buffer (pH 7.0) at 4°C for 3 weeks. Serotyping was performed as described earlier (5).

Fecal samples were also cultured for *Salmonella*, *Shigella* and *Campylobacter* species by standard microbiologic methods.

Serology

Agglutinins. Serum samples were titrated against unheated suspensions (OD, MacFarland standard 5) of *Y. enterocolitica* serotype O3, O5.27, O6.30, O7.8, O8 and O9 and *Yersinia pseudotuberculosis* serotypes I–VI grown at 22°C for 48 h, with a standard agglutination test (2, 5). A titre of antibody $\geq 1:160$ was considered significant. Positive, acute phase sera from patients with positive cultures and agglutinins-negative sera were used as controls in each test run.

IgA and IgG antibodies against Yops. Specific IgA and IgG antibodies against purified plasmid-encoded virulence-associated Yops of *Y. enterocolitica* serotype O8 (strain WA-314; Dr C. Pai,

Table I. Demographic data of 261 patients with enteric forms of yersiniosis

Disease	No.	Age range (years)						Sex M/F	Hospitalized patients
		0-5	6-10	11-15	16-25	26-40	> 40		
Enteritis	169	60	10	12	22	32	33	84/85	33
Enteritis + complications	37	5	1	1	9	12	9	15/22	13
Appendicular syndrome	33	1	5	9	10	5	3	13/20	33
Ileitis	8	—	—	1	1	4	2	2/6	8
Colitis	14	—	—	—	5	4	5	10/4	14

Montreal, Canada) in sera were demonstrated by immunoblotting techniques (4).

Antigens (molec wt: 25 kDa = Yop E, 36 kDa = Yop D, 38 kDa = V-antigen, 46 kDa = Yop H and 58 kDa = Yop M) were blotted onto a nitrocellulose filter (Sigma Chemical Co, St. Louis, MO, USA). Sera were diluted 1:100 in PBS-Tween (150 mM NaCl, 20 mM Na₂HPO₄ (pH 7.0) and 0.5% Tween-20) and incubated with the antigen-coated nitrocellulose strips overnight at 22°C. The IgG and IgA antibody-antigen complexes formed were visualized according to the methods of Blake et al. (10). Control strips were reacted with human acute sera (culture-positive *Y. enterocolitica* infection) containing antibodies to the Yops and human negative sera in each test run. Positive reaction (IgG and IgA) in immunoblotting with at least two identical Yops was judged significant.

Antigen detection in biopsies

Biopsy specimens (enteric preparations) from patients were investigated with serotype-specific (O3, O5, O8, O9) and monospecific (Yad A and Yop H) rabbit antisera for the presence of *Y. enterocolitica* bacilli by indirect immunofluorescence (5). Positive controls consisted of tonsillar tissue contaminated with each of the same serotypes of *Y. enterocolitica*.

Yersinia pseudotuberculosis; *Salmonella typhimurium*; *Shigella sonnei*; *Campylobacter jejuni* and mixtures of aerobic and anaerobic fecal flora were used as negative controls. Rabbit antisera, in dilutions of 1:120–1:900, were added, followed by addition of FITC-conjugated horse antiserum to rabbit IgG. The preparations with *Y. enterocolitica* showed a clear, sharp fluorescence of the cell wall. The reaction was serotype specific at dilutions $\geq 1:240$; at lower dilutions slight cross-reactions were seen between the various serotypes. All negative controls gave no reaction.

Patients

Patients were identified from specimens sent to the Public Health Laboratory in Friesland (The Netherlands). This laboratory serves a province of 700,000 inhabitants with 8 general hospitals (2,100 beds) and 260 general physicians. The study period was from 1 July 1982 to 1 January 1991. Because specificity of the agglutination test is low and there is little information on specificity of the immunoblotting only patients who were culture-positive or had at least two of the other tests (agglutination, immunoblotting, antigen detection) positive, when culture-negative were included. *Yersinia enterocolitica* infection was diagnosed in 473 patients. A physician was asked to observe each patient with a proven *Yersinia* infection for at least 2 months to obtain information on specific symptoms [diarrhea (with or without blood), fever, nausea, vomiting, abdominal pain, weight loss or other], complications (lymphadenitis, arthritis, erythema nodosum or other), clinical course, duration of disease, family history, use of antibiotics, effect of treatment and outcome, underlying disease, drug abuse. A standard form to

record all data was used. Complete record forms were obtained from 403 patients. The patients with gastrointestinal disease ($n = 261$) are presented in this study. The other patients ($n = 142$) had complicated yersiniosis such as arthritis and erythema nodosum without gastrointestinal symptoms and will be reported separately.

The patients were arbitrarily classified into 4 groups: (1) enteritis with or without complications: patients starting with mild diarrhea and abdominal pain; (2) appendicular syndrome: those with acute abdominal pain in the right lower quadrant; (3) ileitis: patients with inflammation of the ileum confirmed by endoscopy; and (4) colitis: patients with inflammation of the colon confirmed by endoscopy.

Statistical analysis

The χ^2 test was used to assess the significance of differences between groups; in the case of small numbers the Fisher's exact test was used. p values are two-tailed.

RESULTS

Incidence

Yersinia enterocolitica was found in 2.4% of the fecal samples, *Salmonella* in 7.8%, *Campylobacter* in 4.6% and *Shigella* in 0.2%. Mixed infections were diagnosed in 0.3% (*S. typhimurium* and *Y. enterocolitica*) and 0.1% (*C. jejuni* and *Y. enterocolitica*), respectively. The patients with the mixed infections were excluded from the study. *Yersinia* infections showed no seasonal peak.

Clinical manifestations

Correlation between clinical manifestations, age, sex and hospitalization is presented in Table I. Two patients had an underlying disease (rectal carcinoma and non-Hodgkin's lymphoma, respectively). None of the patients was known to have abused alcoholic or used iron supplements.

Enteritis. Enteritis was diagnosed in 206 patients. Uncomplicated enteritis (169 patients) was relatively common in children with the highest incidence under the age of 2 years. A mild nonbloody diarrhea was the most common symptom. The duration before diagnosis was 1 week or longer if the diarrhea was relapsing; 34 patients had had periods of diarrhea for 2–3 months, whereas 6 had a prolonged course of up to 1 year afterwards. An acute onset was mentioned by 22%. Abdominal pain occurred more often in adults (≥ 18 years, 64%) than in children (41%), vomiting more often in children (26%) than in adults (≥ 18 years,

Table II. Percentage of positive diagnostic methods related to clinical disease. The number of patients, positive/total tested is in parentheses

Disease	Percentage of positive test				
	Enteritis		Appendicular syndrome (n = 33)	Ileitis (n = 8)	Colitis (n = 14)
	uncompl (n = 169)	compl (n = 37)			
Method					
Culture	99 (162/164)	66 (21/32)	80 (16/20)	13 (1/8)	50 (7/14)
Agglutinins	48 (11/23)	42 (11/26)	32 (7/22)	38 (3/8)	31 (4/13)
Immunoblot	88 (14/16)	92 (22/24)	100 (21/21)	100 (8/8)	80 (8/10)
Antigen detection	ND	94 (15/16)	100 (18/18)	100 (7/7)	91 (10/11)

12%). Fever (31%) and weight loss (19%) were seen equally in all age groups. A total of 26 children and 7 adults needed hospitalization.

Yersinia-associated complications were observed in 37 patients. They included septicaemia (n = 3), generalized lymphadenitis (n = 5), localized lymphadenitis (mesenterial and inguinal, n = 4), arthritis (n = 15), erythema nodosum (n = 8) and disturbed liver functions (n = 7). Four patients had more than one complication. The patients with complications presented more often with fever (52%) than those with uncomplicated enteritis (31%). Antimicrobial treatment was given to 38% of the patients with uncomplicated enteritis and to 75% with complicated enteritis. Various antimicrobial agents, including trimethoprim/sulfamethoxazol and ciprofloxacin, were used. Overall cure rate after 2 months was 93% for uncomplicated yersiniosis and 71% for complicated forms; 17 (of 206) patients still complained of relapsing diarrhea up to 1 year afterwards.

Appendicular syndrome. Presented as 'acute abdomen', occurring in 33 patients, predominantly in young adults. Fever was observed in 36% and 30% had diarrhea. 15 patients had a history of chronic abdominal pain with weight loss (up to 15 kg in 6 months). 20 patients underwent laparotomy; mesenteric lymphadenitis with terminal ileitis with a normal appendix was found in 50% and a slightly inflamed appendix in the others. Four patients had extensive intra-abdominal lymphadenitis with pseudotumorous forms, 1 patient also had cholecystitis due to Y. enterocolitica. One of the patients developed generalized peritonitis and died. Cure without complications was achieved in 25 patients, 8 patients complained of abdominal pain after surgery during the follow-up for 1–12 months.

Ileitis. Observed in 8 patients, 1 child (15 years) and 7 adults. They suffered from chronic relapsing, sometimes bloody diarrhea with abdominal pain, fever and weight loss. The duration of symptoms at the time of diagnosis varied from 6 weeks to 3 years. At endoscopy the intestinal wall appeared diffusely swollen and erythematous with

signs of chronic inflammation. Three patients underwent ileocecal resection. At operation, extensive intra-abdominal lymphadenitis was found in 2 patients; 1 patient also had generalized peritonitis. The ileal resections showed inflammation of submucosa and muscular areas with lymphocytic infiltrates, necrosis and sometimes granulomas. No giant cells or fistulae were observed. Yersinia-associated complications were reactive arthritis (n = 2) and erythema nodosum (n = 2). Four patients were treated with antimicrobial agents (cotrimoxazole or doxycycline), 3 of them were cured and 1 relapsed. The other 4 patients became asymptomatic without therapy.

Colitis. Severe enterocolitis was observed in 14 adults, 10 of them suffering from an acute generalized form; 4 had had a relapsing form of a more localized proctitis for 2 years. Patients with the acute form were very ill with high fever, bloody liquid stools and dehydration. On endoscopy, diffuse ulceration and inflammation of ileum and colon with perforation were observed; 5 patients had toxic megacolon. Three patients died despite antimicrobial treatment (cefoperazone + gentamicin + metronidazole, doxycyclin, cefuroxim) and surgery. The other 7 patients with the acute form were cured with antimicrobial treatment (cotrimoxazole or ciprofloxacin or doxycyclin) and surgery. One of the 4 patients with the more localized relapsing form still had symptoms during the follow-up despite antimicrobial treatment (ciprofloxacin).

Yersinia-associated complications were: arthritis (n = 6) and erythema nodosum (n = 1). Three patients showed disturbed liverfunctions.

Microbiology

Table II gives the results of diagnostic methods related to clinical presentation. Virtually all patients with uncomplicated enteritis from whom feces was cultured were culture-positive (99%), as were 80% of the patients with an appendicular syndrome and less than 50% of the patients with ileitis and colitis.

The strains belonged to serotype O3 (97 strains), O9 (49 strains), O8 (20 strains), O5.27 (13 strains), O7.8 (12

strains), O6.3 (4 strains) and not typeable (12 strains). Serotypes O3 or O9 were present in 90% of the culture-positive patients younger than 15 years but in only 64% of patients older than 15 years. At least 2 patients excreted *Y. enterocolitica* (both O3) for more than 2 months. None of the patients with colitis had O3 or O9 strains.

From 1982 until 1986 90 patients were included. After introduction of immunoblotting and antigen detection 171 patients were recognized. Blood or biopsy specimens available from patients with yersiniosis diagnosed before 1986 were also tested retrospectively with the new techniques.

In 50 of the 54 culture-negative patients both agglutination and immunoblotting were performed. Only 17 of 50 patients with positive immunoblotting were also agglutinin positive ($p < 0.0001$).

By antigen detection, *Yersinia bacilli* (5 or more per high power microscopic field) were found in or between the epithelial cells, in the infiltrates and lymphoid tissue. A total of 40 biopsy specimens were cultured but only 1 was positive. A second biopsy after 6 months was taken from 8 patients. They were found positive in 5 patients.

DISCUSSION

Yersiniosis is a rather common disease in The Netherlands. The incidence seems rather high compared with most European countries (11). However, in Belgium an isolation rate of 5.9% has been reported previously (12). Epidemiology and transmission were not investigated in this study, although we observed some families with more than 1 case.

The incidence of uncomplicated enteritis was highest in young children. It is probable that the incidence in this age group is even higher, as it is often a mild, self-limiting disease at that age. In this study bloody stools were an exception in young children. Bloody stools in children are found in variable percentages (16–29%) (13, 14). Most of our children were seen by general practitioners in an outpatient setting as we reported earlier (15).

The long duration of the diarrhea in 25% of the patients with enteritis was remarkable. Reports from Norway also mention the chronicity of *Yersinia enteritis* (14). The more generalized and complicated course of *Yersinia enteritis* was mainly seen in adults (≥ 18 years, 34%) and not in children (11%). This observation is in contrast with that reported by others (16) who found complications in up to 7%, but fits well with the observation of Orloff et al. (14) who showed joint pain to be a frequent complication (52%) in adults with enteritis. Abdominal pain is a cardinal feature of *Yersinia enteric* infections, with pseudoappendicular syndrome the most prominent and classic. It was the reason for emergency surgery in 20 patients and like others (17) we experienced that surgery was not efficacious in all patients.

The tendency to chronicity was also a cardinal feature, especially in patients with ileitis, which presented as a

severe illness resembling Crohn's disease and affected only adults. Our patients had the more chronic form, whereas others (17, 18) found only terminal ileitis associated with acute pseudoappendicular syndromes. Most of the patients with chronic forms had negative fecal cultures. After the acute phase organisms migrate to the GALT (Gut Associated Lymphoid Tissue) and into the mesenteric lymph nodes, which we can confirm by antigen detection in biopsy specimens, and are not excreted anymore. Circulating agglutinins are known to disappear after the acute phase, and the agglutinin test will then become negative (2). For these patients the new tests including demonstration of antiYops were essential in establishing the correct diagnosis. One may speculate that a number of patients diagnosed as having idiopathic ileitis may in fact suffer from chronic *Y. enterocolitica* infection.

This may also be the case for a number of patients with 'idiopathic' colitis. We observed two forms: a very severe, acute fulminant form ($n = 10$), which was fatal in 3 patients and a more chronic 'ulcerative colitis' form in 4 patients. Like others we found colitis restricted to adults (19).

Yersinia-associated complications were found especially in adult patients with chronic rather than acute forms. Both female and male were equally affected by all forms of complications, except for erythema nodosum which was found predominantly in women (9 females vs 2 males, $p = 0.06$). Persistence of infection may be responsible for or involved in these complications (3). Age may be an important determinant for the seriousness of the course of disease, as we found that yersiniosis at an older age often had a complicated course. So far, no satisfactory explanation for this phenomenon has been found.

The strains cultured in this study belonged to the serotypes commonly in Europe (5, 9). We also isolated 20 strains which only reacted with antiserum against serotype O8, a type that generally is not present in Europe. We suggest that this type shows high similarity and therefore cross-reacts with the American O8 strain (5) as was also described in other European strains (20). 29 strains did not belong to the serotypes O3, O8 or O9. The pathogenic potential of such strains is controversial, but several reports have been published on the relationship between these 'unusual' serotypes or biogroups and clinical disease, even with fatal outcome (21–23). We isolated unusual serotypes more often from older patients and especially from patients with colitis. Similarly Capriolo et al (21) observed that 'unusual' serotypes were associated with advancing age. We have demonstrated previously that 15–20% of young healthy adults in endemic areas have circulating IgG specific to 1 or 2 Yops (24), reflecting contact with virulent *Yersinia* strains in childhood. These antibodies may protect against reinfection by common types at an older age. This could explain the shift to 'unusual' serotypes at an older age. Further studies are needed to elucidate the pathogenicity of unusual serotypes.

Antimicrobial treatment does not seem to influence the course and duration of illness in uncomplicated enteritis but an effect on shedding of the organism has been described (14). However the effect of antibiotics on complicated yersiniosis is still not clear. The frequency and seriousness of these complications require controlled trials with various antimicrobials.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr J. Heesemann and Mrs I. Guicherit for performing immunoblotting tests, Dr J. de Koning for histology, all colleagues who delivered patients' data, Dr J. Meis and Dr H. Muytjens for reviewing the manuscript.

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Submitted March 3, 1996; accepted August 7, 1996